ALTERED COAGULABILITY: AN AID TO SELECTIVE BREAST BIOPSY

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Difficulty in discriminating nonadvanced breast cancer from benign breast disease results in many cancer negative biopsies. Development of a test to better differentiate between these two entities to reduce the number of cancer negative biopsies was the purpose of this blind study. The clue that prompted the development of this test resides in the state of hypercoagulability in cancer. Hypercoagulability can be measured by assessing tissue factor-mediated altered coagulability. The amount of tissue factor release is contingent on prior activation of the monocyte (the only blood cell that generates tissue factor) in vivo. (*J Natl Med Assoc.* 1993;85:273-277.)

Key words • breast cancer • hypercoagulability • tissue factor • fibrocystic breast disease

Histopathologic verification by open biopsy is the most reliable means for confirming the diagnosis of carcinoma of the breast.[1] However, because of the low biopsy positive yield of mammographically labeled malignancy and because of the overlap in the clinical mammographic findings between breast cancer and fibrocystic disease, a variable but significant number of cancer-negative biopsies are performed.[2] Hitherto, there has been no test or combination of tests to discriminate between nonadvanced breast cancer and fibrocystic disease.[3] This article describes the rationale, methodology, and application of a test that can achieve this objective by detecting the presence of altered coagulability.

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RATIONALE

The clue that prompted the development of this test is the state of hypercoagulability present in patients with cancer, which was first documented by Armand Trousseau[4] more than 130 years ago. This phenomenon has remained dormant because of the lack of an appropriate coagulation test to prove the association between cancer and this state of hypercoagulability.

The test described here is based on the most important but poorly appreciated contributory role of the monocyte-generated procoagulant material, tissue factor, also referred to as thromboplastin, in the extrinsic pathway of the coagulation process.[5] The monocyte is constantly prone to release this procoagulant tissue factor when activated by endogenous (eg, cancer) or exogenous (eg, infection via endotoxin) influences.[6,7] This is essentially a functional property of the monocyte as the total monocyte count remains within normal limits during this episode of activation.

Techniques for demonstrating this property of the monocyte have been laborious, expensive, and unadaptable to routine clinical application. Osterud and Bjorklid[8] devised a simple technique to evaluate this property of the monocyte. Incubation of citrated blood with endotoxin at increasing concentrations for increasing periods of time results in proportionately reduced recalcification times (RT) that reflect hypercoagulability due to tissue factor generation.

Many disease states are associated with hypercoagulability that may be related to the generation of monocyte tissue factor activity when compared with controls.[9-19] Because the monocyte is the only circulating cell capable of generating significant tissue factor activity,[20] addition of endotoxin to whole blood results in varying degrees of clottability directly related to the amount of tissue factor released.

It should be emphasized that the degree of tissue factor activity is also directly related either to transient

TABLE 1. RECALCIFICATION TIMES OF THE ENDOTOXIN-INCUBATED SAMPLE (RTE) FOR BREAST CANCER AND BENIGN BREAST DISEASE PATIENTS

	No. of Patients	RTE (Minutes)		
		Median	Mean	SD
Nonadvanced cancer	27	3.6	3.52	.62
Advanced cancer*	11	4.8	5.02	.76
Benign breast disease†	92	4.4	4.41	1.07

Abbreviations: SD = standard deviation.
*$P = .0001$.
†$P = .001$.

or to persistent prior activation of the monocyte in vivo.[6] Therefore, the hallmark of the test is the variation in tissue factor production depending on the duration and intensity of these stimuli. The effects of tissue factor on coagulability can be quantitated by measuring the RT with numerical precision using the Sonoclot Coagulation Analyzer (Sienco Inc, Morrison, Colorado).

METHODS

One hundred thirty women, ages 16 to 73 years, who were advised to undergo excisional breast biopsy on the basis of clinical and/or mammographic criteria, comprised the study population. Pregnant women were not included in the study population because this condition has been shown to affect the RT values.[12] Aliquots of atraumatically obtained citrated venous blood (1 mL) were added to 20 μL of saline containing 10 μg of *Escherichia coli* endotoxin (055:B5W No. 3120-25, Difco, Detroit, Michigan). The samples were incubated at 37°C for 2 hours, then 40 μl of 0.1 M $CaCl_2$ was added to 0.3 mL of the incubated blood, to initiate the process of clotting, and the RT of the endotoxin-incubated samples (RTE) were obtained. Each test was performed in a blinded manner (ie, without knowledge of the biopsy result). If the RTE test was repeated within a period of 3 months, the average values were reported.

For the purposes of evaluation of the RTE, the histopathologic diagnosis of breast lesions is considered to be the "criterion standard" and the sensitivity, specificity, and predictive value of a positive test result, and the predictive value of a negative test result, therefore, were calculated with confidence intervals on these proportions.

RESULTS

Biopsies of the 130 patients revealed 38 patients with breast cancer and 92 with benign breast disease, which included fibrocystic disease (69 patients), fibroadenoma (9 patients), intraductal papilloma (6 patients), and miscellaneous entities (8 patients). Among the cancer patients, there were 27 with nonadvanced cancer (including 3 with in situ, noninfiltrating intraductal carcinoma and 11 with advanced cancer as determined by clinical or radiological evidence of extensive local disease or distant metastases). The RTE values are shown in Table 1. The RTE values were considerably shorter in the nonadvanced cancer patients compared with those patients with advanced disease ($P = .0001$) and all the nonadvanced cancer patients had RTE values shorter than 4.4 minutes. Similarly, the RTE values were shorter in the nonadvanced cancer patients compared with those patients with benign breast disease ($P = .001$).

Using a 4.4-minute RTE value as the best criterion to detect nonadvanced cancer (n = 119), the sensitivity of the RTE is 27/27 or 100% (lower 95% confidence interval [CI_{95}] = 87%), the specificity is 44/92 or 48% (CI_{95} = 37% to 58%), the predictive value of a positive test result is 27/75 or 36% (CI_{95} = 25% to 48%), and the predictive value of a negative test result is 44/44 or 100% (lower CI_{95} = 92%).

Comparative diagnostic results between mammography and RTE are depicted in Tables 2 and 3. In order to divide mammographic interpretations into two broad groups (cancer and noncancer), the concluding statements on the mammographic report, as to whether surgical consultation was advised (cancer), or whether to repeat the mammography a year or more later (noncancer), were used. For the 91 women for whom mammography, RTE, and biopsy data were available, the RTE appears to be a superior diagnostic test. The sensitivity, specificity, and predictive values of the RTE all exceeded those for mammography. Mammography tests produced 11 false-negative results (compared with none produced using RTE), and it also produced a higher number of false-positive results than RTE (53 versus 37).

Furthermore, had the RTE been used as a screening test and only those patients with a value of 4.4 or lower underwent mammography, 26 biopsy-proven negative individuals with RTE >4.4 might not have been labeled with a false-positive mammography result, and therefore recommended for biopsy (Table 3). Demographic variables such as age and race were not found to be predictive of RTE values.

TABLE 2. COMPARISON OF MAMMOGRAPHY AND RECALCIFICATION TIMES OF THE ENDOTOXIN-INCUBATED SAMPLE (RTE) AS SCREENING TESTS FOR BREAST CANCER

	Nonadvanced Cancer	Benign Breast Cancer	
Mammography*			
Cancer positive	12	53	65
Cancer negative	11	15	26
	23	68	91
RTE†			
≤4.4	23	37	60
≥4.4	0	31	31
	23	68	91

*Sensitivity = 12/23 = 52% (CI_{95} = 31% to 73%) and specificity = 15/68 = 22% (CI_{95} = 13% to 34%). Predictive value of a positive test result = 12/65 = 18% (CI_{95} = 10% to 30%). Predictive value of a negative test result = 15/26 = 58% (CI_{95} = 37% to 77%).

†Sensitivity = 23/23 = 100% (CI_{95} lower limit = 85%) and specificity = 31/68 = 46% (CI_{95} = 33% to 58%). Predictive value of a positive test result = 23/60 = 38% (CI_{95} = 26% to 52%). Predictive value of a negative test result = 31/31 = 100% (CI_{95} lower limit = 89%).

TABLE 3. MAMMOGRAPHY CLASSIFICATION OF NONADVANCED BREAST CANCER AND BENIGN BREAST DISEASE PATIENTS BY RECALCIFICATION TIMES OF THE ENDOTOXIN-INCUBATED SAMPLE (RTE)

Mammography	Nonadvanced Cancer	Benign Breast Disease	
RTE ≤4.4			
Cancer positive	12	27	39
Cancer negative	11	10	21
	23	37	60
RTE ≥4.4			
Cancer positive	0	26	26
Cancer negative	0	5	5
	0	31	31

DISCUSSION

The RTE "break point" (the longest RTE value for nonadvanced cancer) used in this study was based on previous work[10] as well as on examination of the current study population. While the RTE break point value of 4.4 is somewhat arbitrary, the use of this break point presents rather dramatic results: higher levels of sensitivity, specificity, and predictive value than for mammography in this group of women. Because the study population does not represent a screening population, an attempt should be made to replicate these findings using a large group of women who are part of an established screening program. In this way, the break point might be further refined, and comparable screening program test values can be calculated for mammography and RTE.

The data obtained in this study clearly demonstrate the significant shortening in the RTE values resulting from a state of hypercoagulability in patients with nonadvanced breast cancer compared with those with advanced breast cancer. Prolongation of the RTE in advanced breast malignancy is of interest but is of no diagnostic concern, since, in advanced cancer, the stigmata of the magnitude of local disease and distant metastases are usually clinically evident and easily verifiable histologically. Why the RTE is prolonged when the cancer reaches a later stage is unknown but is in keeping with the existence of a bleeding diathesis in such patients.[21]

The most exciting and striking data concerning the RTE obtained in this study are the superior sensitivity, specificity, and predictive values of the RTE test result when compared with mammography. If these findings are replicated in other populations, the RTE can, with reliability, aid in selecting those patients with suspicious mammographic interpretations who have RTE readings <4.4 minutes for confirmatory excisional biopsy. Also, patients with RTE readings >4.4 minutes, in the absence of signs of advanced breast cancer or a palpable solid mass, can be considered to have benign breast disease and be exempted from biopsy at this time. This observation concerning the ability of the RTE to strongly support the diagnosis of nonadvanced malignant disease, to negate the possibility of nonadvanced cancer, and to indicate the presence of benign breast disease fulfilled the objective of this study. Hence, RTE has the potential to become the most dependable method to negate false-positive mammographic interpretations and thus avert all the adverse consequences of a false-negative breast biopsy.

At present, because of the time it takes for a lesion to become palpable, the early detection of breast cancer depends almost entirely on mammography. However, wide variation in the positive findings of nonpalpable lesions in patients undergoing screening mammography

makes it a sensitive but nonspecific test for breast cancer.[2] Also, even when such nonpalpable lesions are detected by this method, some already show lymph node metastasis.[22] This is further exemplified by the data noted in two mass mammography screening programs where more than 20% of breast cancers that were detected were associated with nodal metastases.[23,24]

A thought-provoking finding was a (nonadvanced) cancer-positive RTE associated with in situ noninfiltrating intraductal cancer. This may be the most important revelation in this study. Apart from assisting in the diagnosis of cancer at the earliest possible time, the RTE may provide that long awaited signal that can pinpoint almost the precise moment when anticancer defenses abandon their responsibility and succumb to the unrelenting onslaught of unhampered cell division, the very essence of the malignant process. This signal, if proven to be reliable, can provide the "open sesame" to vital basic investigations that can easily be incorporated into the routine breast screening maneuvers.

In our study, fibrocystic disease is the predominant contender in the differential diagnosis of breast lesions. Patients with benign breast disease (mostly fibrocystic disease) had a wide range (difference between the highest or lowest value of RTE times) of 4.6 minutes with a mean of 4.5 minutes. These figures are significantly longer than in the nonadvanced cancer patients who had an RTE range of 2.4 minutes and a mean of 3.5 minutes. These data authenticate the ability of the RTE to differentiate fibrocystic disease from nonadvanced cancer. Therefore, patients with RTE values >4.4 minutes can be managed conservatively by periodic physical examinations, RTE tests, and mammography. The remaining patients with fibrocystic disease who had RTE values <4.4 minutes warrant closer scrutiny at regular intervals for evidence of breast cancer. Because some patients in the latter group will be destined to develop breast cancer, it is conceivable that the low RTE values seen in them may herald such a development.

In this study, no attempt was made to exclude factors that would affect the validity of the RTE results. The only apparent condition that rendered the test hypercoagulable was found, retrospectively, to be pregnancy. Shortened RTE values in pregnant patients with benign breast disease were not surprising. In one earlier study,[12] we have shown that a state of hypercoagulability persists through the three trimesters of pregnancy and continues well beyond the puerperium. The four pregnant women were, therefore, excluded from these analyses.

In the interpretation of the RTE, it should be emphasized that other stimuli can affect these test results. If serial values revert to the normal range, it would imply that the incriminating stimuli are transient and may include factors such as strenuous physical activity or a possible short-lived viral or bacterial infection. Repeated short RTE values are to be viewed more cautiously and would strongly recommend systematic investigation of the patient for more persistent stimuli generated by breast and other cancers, by diabetes, and by other established hypercoagulable conditions.[9-19]

Any possible changes in the RTE following surgical excision of the primary lesion were not routinely monitored because patients with both early and late breast cancer were subject to radiotherapy or chemotherapy. Because these adjuvant therapeutic modalities are known to affect immune function, and because the monocyte is an important component of the immune system, it was felt that any RTE values obtained under these circumstances would be questionable. Nevertheless, a much earlier study conducted in an animal model has shown that the hypercoagulable state is abolished by complete removal of the tumor, but the state persists if any residual tumor is present or if a "sham" excisional operation is performed.[25,26] Such a study would not be ethically permissible in humans as this would deny such patients appropriate adjuvant therapy.

We did not, in the presence of a positive RTE, pursue efforts to incriminate cancers of other organs or a variety of other disease processes. This decision was in compliance with the Institutional Review Board proviso that the RTE should not dictate any therapeutic measures.

The adverse effects of false-positive biopsies have received little or no attention. The psychological impact on the woman resulting from apprehension of the impending operation added to the universal fear of cancer, the dread of surgical trauma to the breast, and repercussions on the family cannot be adequately described. The risk of surgical complications is low, but significant. Among the most serious of these are anesthetically potentiated cardiac problems that can be life-threatening and wound infection that can lead to considerable disfigurement of the breast. Of alarming medicolegal concern is a false-positive or false-negative histopathologic diagnosis due to errors on the part of the surgeon (for excising the wrong tissue) or the pathologist (for examining the wrong tissue or for misdiagnosing the right tissue).

Financially, the high cost of negative biopsies is

seldom considered. While the exact monetary figure of all these cancer-negative biopsy procedures performed each year is difficult to compute, the magnitude of the economic burden can be exemplified by estimates derived from a representative sample. If the cost of the biopsy is about $5000 (operating room and recovery room charges, fees for radiologist, surgeon, anesthesiologist, pathologist, laboratory tests, etc), in a population of 100 000 women, the total cost is a staggering $500 million per annum. This expenditure must be recovered by taxes to support federal contributions (Medicaid and Medicare) and by increased insurance premiums.

Mammograms are not generally performed or recommended more frequently than at yearly intervals. In contrast to mammography, the RTE can be performed as frequently as deemed advisable, the only limitations being inconvenience to the patient and cost. The RTE test, from the standpoint of cost, is economically feasible. Being a coagulation test, most, if not all, health insurance programs should absorb the charge, if reasonable.

Before the introduction of any new test, clinical need must first be established and the cost-benefit ratio assessed. From the evidence provided in this report and based on efficacy, cost, and simplicity, the RTE test warrants urgent multicenter assessment as augmentational to customary breast screening procedures of breast examination and mammography.

Literature Cited

1. Scanlon EF. The breast biopsy. *Cancer.* 1989;64:25671-25672.
2. Erickson EJ, McGreevy JM, Maskett A. Selective nonoperative management of patients referred with abnormal mammograms. *Am J Surg.* 1990;160:659-664.
3. Shroff JH, Lloyd LR, Schroder DM. Open breast biopsy: a critical analysis. *Am Surg.* 1991;57:481-484.
4. Trousseau A. Phlegmasia alba dolens. In: *Clinique Medicale de L'Hotel-Dieu de Paris.* 2nd ed, vol 3. Paris, France: JB Bailliere; 1865:654-712.
5. Morgan D, Edwards RL, Rickles FR. Monocyte procoagulant activity as a peripheral marker of clotting activation in cancer patients. *Haemostasis.* 1988;18:55-65.
6. Marx JL. Coagulation as a common thread in disease. *Science.* 1982;218:145-146.
7. Rivers RPA, Hathaway WE, Weston WL. The endotoxin-induced coagulant activity. *Br J Haematol.* 1975;30:311-316.
8. Osterud B, Bjorklid E. The production and availability of tissue thromboplastin in cellular populations of whole blood exposed to various concentrations of endotoxin. *Scandinavian Journal of Haematology.* 1982;29:175-184.
9. Spillert CR, Hafstein MP, Lanka V, Lazaro EJ. Increased thromboplastin production in multiple sclerosis: an immunologic defect? *Ann NY Acad Sci.* 1986;475:345-346.
10. Spillert CR, Lazaro EJ. Shortened endotoxin-activated clotting times in patients with carcinoma. *Am Surg.* 1987;53:164-166.
11. Dasmahapatra KS, Cheung NR, Spillert CR, Lazaro EJ. An assessment of monocyte procoagulant activity in patients with solid tumors. *J Surg Res.* 1987;43:158-169.
12. Dispenziere BH, Spillert CR, Lazaro EJ. Assessment of hypercoagulability in pregnancy. *Clin Res.* 1988;36:409A.
13. Sophia CA, Spillert CR, Pastena JA, Lazaro EJ. Accentuated recalcification time in breast disease. *J Natl Med Assoc.* 1988;80:1214-1216.
14. Spillert CR, Curtis GT, Lazaro EJ. Assessment of monocyte function in the diagnosis of breast disease. *South Med J.* 1988;81:164-166.
15. Spillert CR, Milazzo JT, Suval WD, Lazaro EJ. Hypercoagulability in arterial disease. *Angiology.* 1989;40:886-889.
16. Spillert CR, Milazzo VJ, Pastena JA, Lazaro EJ. Prediction of clinical course in diabetes using a simple coagulation test. *J Natl Med Assoc.* 1989;81:81-83.
17. McCullough JN, Spillert CR, Lazaro EJ. A direct correlation between injury severity and two markers of the functional status of the immune system. *Circ Shock.* 1990;31:309-316.
18. Spillert CR, Lazaro EJ. Contribution of the monocyte to thrombotic potential. *Agents Actions.* 1991;34:28-29.
19. Spillert CR, Suval WD, Lazaro EJ. Assessment of immune competence in postsurgical and post-traumatic patients. *Surgical Research Communications.* 1991;10:31-34.
20. Dean RT, Leoni P, Rossi BC. Regulation of procoagulant factors in mononuclear phagocytes. *Haemostasis.* 1984;14:412-421.
21. Edwards RL, Rickles FR, Cronlund M. Abnormalities of blood coagulation in patients with cancer. *J Lab Clin Med.* 1981;98:917-928.
22. Sener SF, Candela FC, Paige ML, Bernstein JR, Winchester DP. Limitations of mammography in the identification of noninfiltrating carcinoma of the breast. *Surg Gynecol Obstet.* 1988;167:135-140.
23. Roberts MM, Alexander FE, Anderson TJ, Chetty V, Donnan PT. Edinburgh trial of screening for breast cancer: mortality at seven years. *Lancet.* 1990;335(8684):241-246.
24. Nesin N, Elliott BA, Elliott TT. Screening mammography in a primary case setting. *Minn Med.* 1988;71:209-212.
25. Karanfilian RC, Spillert CR, Raina S, Lazaro EJ. Accelerated coagulation in a non-metastasizing tumor in rats. *Curr Surg.* 1981;38:385-387.
26. Raina S, Spillert CR, Greenstein SM, Lazaro EJ. Effect of surgery on tumor-induced accelerated coagulation in a rat carcinoma. *J Surg Res.* 1985;38:138-142.